Emirates Journal of Food and Agriculture. 2023. 35(7): 620-630
doi: 10.9755/ejfa.2023.v35.i7.3112
http://www.ejfa.me/

RESEARCH ARTICLE

Purification and characterization of acidophilic xylanase from *Bacillus sp.* with potential application as fruit juice clarifier

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ABSTRACT

Two bacterial isolates from the mangrove's soil were isolated and screened for xylanase production. Screened isolates designated as AS1 and AS2 were identified by sequencing and found to be *Bacillus altitudinis* and *Bacillus licheniformis*. The highest xylanase activity recorded from *Bacillus altitudinis* is 82.664 units per ml and from *Bacillus licheniformis* is 153.59 unitsper ml at pH 4.0. Purification of the crude enzyme was done by molecular sieve chromatography and molecular weight was estimated by the SDS PAGE method. The molecular weight of xylanase was noted as 43 and 40 kDa respectively. Temperature 40°C and pH 4.0 was found to be optimum for purified xylanase enzyme. Purified xylanase was active in broad pH and temperature range. Purified xylanase activity was repressed upon incubation with FeSO4 solution. Application of purified xylanase was done in clarifying different fruit juices.The results of fruit juice clarification indicate the escalation in the sugar concentration and transparency in the juices. Percent transmittance of fruit juices showed 25.11%, 75.82%, 52.40%, 40.09%, and 30.21% for apple, orange, mousambi, pineapple, and kiwi respectively, with *Bacillus altitudinis* xylanase. while 44.31%, 80.12%, 72.11%, 33.00%, and 21.42 for apple, orange, mousambi, pineapple, and kiwi respectively, with *Bacillus licheniformis* Xylanase. The application studies show the potential of purified xylanase as a clarifier in food Industries.

Keywords: Acidophiles; Xylanase; Fruit juice clarification; Profiling; *Bacillus sp*

INTRODUCTION

Hemicellulose is one of the structurally different and abundant polysaccharide available in the environment (Liao et al. 2014; Mezrlow 2011). It containsxylans,galactans, and mannans.Xylans and mannans are the most widespread and it containarabinans and glucanmannans(Zorec et al. 2014). Xylan represents a major portion of hemicellulose content varying in concentration from 20 to 40%. The major chunk of xylan can be found in the hardwood (>30%) followed by softwood (15-30%) (Zarafeta et al. 2020; Bhushan et al. 2015). Structurally, xylanis made of a chain of beta xylopyranose attached with beta glycosyl bonds made up of sugars and glucoronic acids.With such high structural complexity, complete hydrolysis of xylan is possible with the combination and synergistic activity of many enzymes. Breakdown of the xylan is done by diverse xylanase enzymes including the xylan-hydrolase, betaxylosidase, alphafuranosidase. Key xylan degrading enzymes found is beta xylanohydrolase resulting in complete xylan breakdown (Ping et al. 2018; Mechelke et al. 2017).The beta xylan hydrolase will break the beta xylosidic bonds resulting in short oligosaccharides and xylose like xylobiose.Most of the identified xylanases are considered to be a part of hydrolase (glycosides) families (Chundawat et al. 2011).

In nature, microorganisms are rich sources of xylanase. Different microbial species produce xylanase enzymes, and multiple forms of xylanase enzymes have been detected in several of these microorganisms (Chadha et al. 2019). All these forms of microbial enzymes are varied by their physiological, chemical, and structural properties. Microbes especially different species of bacteria and fungi are well reported for xylanase production and expression (Guan et al. 2016).

Xylanase has potential applications in detergent industries, food industries as well as in poultry industries (Senthilkumar et al. 2005; Dien et al. 2014). Especially in bakery

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Received: 20 September 2022; **Accepted:** 17 June 2023

products, xylanase is used in the improvement of dough quality for the improvement of elasticity and rheology of products (Bajaj andManhas 2012).Xylanase helps in improving the nutritious aspects of various agricultural products.Furthermore, a combination of cellulase and xylanaseenzymes works effectively in lignocellulose hydrolysis for biofuel production (Chakdar et al. 2016; Badejo et al. 2021).

In Industries, robust enzymes are required which can easily tolerate extreme temperature and pH(Polizeli et al. 2005). Considering such application, enzymes are required which can easily tolerate such harsh conditions. Most acidophilic or acid-stable xylanase would be more applicablein the processes requiring a low pH for the minimization of microbial contamination. Xylanase enzymes are required with brilliant high specific activity, pH, adaptability, and thermal stability in harsh processing conditions(Juturu and Wu 2012).

In current years, as a result of the overall consumption of fruit juices, natural juices are mostly preferred by consumers.However, fruit juice extraction is a complex process resulting in a viscous solution. The viscosity is mainly due to the high concentration of carbohydrates mainly polysaccharides (Nagar et al. 2012). One of the alternatives for increasing the efficiency is the use of hydrolysis enzymes like cellulase, xylanase and pectinase. The prerequisite for use of such an enzyme is only its high stability requirements in acidic conditions (Li et al. 2020). One of the major food processing industries are fruit juice manufacturing industries. The prime requirement of fruit juice manufacturing industry is reduction in juice viscosity keeping the taste and quality of juice intact. Fruit juice clarification can be improved using the hydrolytic enzymes (Bhat 2000). Fruit juices are pectin rich however the citrus fruits, pineapple, apple, mousambi, orange and kiwi also constitute of hemicellulose. It is evident fact that xylan represents the major portion of hemicellulose and hence the xylanase represents key enzyme in fruit juice clarification. Using the dynamic properties of the xylanase, researchers have reported application of xylanase enzyme in the fruit juice application (Dhiman et al. 2011; Nagar et al. 2012 and Rosmine et al. 2017).Moreover, xylooligosaccharides obtained by enzyme mediated hydrolysis are the represents the most significant components for improving food quality because of their specific prebiotic characteristics leading to a significant reduction in cholesterol and blood sugar (Samanta et al. 2015).Keeping the above points in view, the objective of the present study isproduction and purification of acidophilic xylanase from two *Bacillus* species isolated from roots of mangroves plants.Further, purified xylanase is characterized and studied as potential fruit juice clarifier.

MATERIALS AND METHODS

Collection of soil samples

Different soil samples from the roots of mangroves plants were collected from Indian Mangrovesnear Narara beach at Jamnagar, Gujarat. Samples were collected in plastic bags and preserved in freezing conditions for further use.

Isolation of acidophilic bacteria

Isolation of acidophilic bacteria was done using the serial dilution technique. Briefly collected samples were diluted and inoculated on Petri dishes containing Norris media. The chemical composition of Norris media was slightly modified with a change in ammonium sulphate and ferrous ammonium sulphate concentration. As a solidifying agent, the gellan gum (2%) was used in media plates and adjusted to different pH 3.0. The inoculated modified media plates were incubated in an incubator for 4 to 8 days at 37°C.

Screening and identification of isolates

Xylanase producing isolates were screened on the Norris media supplemented with beechwood xylan adjusted with pH 3.5. The pure isolates were congo red dye (0.1%) and zone of clearance was observed.

The pure colony was characterized further for its morphological and staining characteristics. For molecular identification, bacterial DNA was pulled outby a DNA isolation kit available commercially from the Qiagenpvt. Ltd. The isolated DNA was sequenced in the sequencing facility at Eurofins Genomics, India. The sequenced data was BLAST using NCBI Blast.

Enzyme production under the liquid conditions

Xylanase production was carried out in Norris broth supplemented with 10% beechwood xylan and pH 3.5. The broth was keptin shaking conditions for seven days at 120 rpmat 37°C. The incubated culture was checked for enzyme production regularly after 24 hours.

Xylanase assay

Xylanase activity was assayed by DNSA (dinitrosalicylic acid). Enzyme activity was measured by mixing 0.5 ml of 1% of xylan solution prepared 0.5 M citrate buffer. The reaction mixture was incubated for 20 minutes at 37°C followed by termination of reaction using 1 ml of DNSA. Enzyme activity was measured at 540nm using a spectrophotometer (Shimadzu 1800, USA). The Enzyme unit is expressed as one microgram of substrate liberation in a minute using standard assay protocol.

Enzyme purification

Purification of Xylanase

Partial purification of the enzyme was performed by the precipitation method (ammonium sulphate). Crude

xylanase enzyme was saturated with 80% ammonium sulphate and allowed to precipitate with constant stirring for 24 hours in freezing conditions. The precipitated sample was dialyzed and checked for enzyme activity and protein concentration (Lowry et al. 1951). Dialyzed enzyme was purified by molecular sieve chromatography using Bio-Gel P60 as matrix.

Molecular weight determination

Molecular weight determination was carried out bydenaturing SDS PAGE gel electrophoresis (Laemmli 1970). 12% resolving gel was used and detection was carried out using the Coomassie brilliant blue staining.

Xylanase characterization

Effect of pH and temperature on xylanase enzyme activity

Purified xylanase was characterized using 0.05 M of citrate buffer (3.0-5.0), phosphate buffer (6.0-7.0), and tris buffer (7.0-8.0) for determining effect of pH on enzyme activity. The enzyme was incubated with different pH buffers and incubated for 20 mins. The effect of temperature was determined by incubating the enzyme at 37°C, 40°C, 50°C, and 60°C for 20 mins. After incubation at a specific temperature and pH buffers, the enzyme activity was measured at 540nm using DNSA method.

Influence of metal ions and chelators

Xylanase activity was measured by incubating the purified enzyme in 5mM and 10mM$CaCO_3$, $FeCl_3$, $CoCl_2$, $MnSO_4$, $MgCl_2$, $ZnCl_2$, $MnCl_2$, $CuSO_4$, $MgSO_4$, $MnSO_4$, $FeSO_4$, $ZnSO_4$, and chelators such as EDTA and SDS. The pure enzyme was incubated with metal ions and chelators for 20 minutes at 37°C. After incubation, xylanase activity was measured using DNSA method at 540nm.

Statistical analysis

For comparing the treatments of different parameters like temperature, pH and metal ions, Duncan's multiple range test was performed. The enzyme assay was done in triplicates and for Duncan's multiple range test the recorded optical density after enzyme assay was considered to find the significant difference between effects of the tested parameters on enzyme activity.

Fruit juice clarification

Total of 5 different fruits viz: Apple (Malusdomestica), Orange (Citrus sinensis), Mousambi (Citrus limetta), Pineapple (Ananascomosus), and Kiwi (Actinidiadeliciosa) were used in the study. Fruits were thoroughly washed, peeled, and the seeds were removed before enzyme treatment. Fruit pulps were extracted using homogenizer. After homogenization, the fruit pulps were processed for a short time pasteurization at 85°C for 5 minutes in a boiling water bath followed by cooling at 40°C. The pulp was separated and the juice was incubated with enzyme for 1 hour, 3 hours, 6 hours and 24 hours of incubation at 37°C. After centrifugation, the resultant supernatant was used to determine the transparency and sugar content. The clarification was by determining (%T) at 660 nm using a UV visible spectrophotometer (Shimadzu 1800).The other parameters like Total soluble solids were expressed in value (°BRIX).

RESULT AND DISCUSSION

Xylanolytic bacteria isolation

On performing isolation, 11 different acidophilic bacteria (AS1 to AS11) were isolated from the mangroves plant of Narara Beach at Jamnagar, Gujarat, India. All 11 isolates were able to grow at pH 4, amongst, 7 isolates that can grow at pH 3.5, were selected for further study. Those 7 bacterial strains were screened for xylanase-producing activity, and the 2 isolates (AS1& AS2) were selected as they produced a significant amount of pH-stable xylanase. The isolated organisms were studied for their colony characteristics and staining behaviors (Fig. 1)

Morphological and physiological characteristics of isolates

The isolates were studied based on morphological and physiological tests. The isolate AS1 has a circular, entire, opaque, and white color colony appearance. AS2 has a rough, irregular, opaque, and pale white pigmented colony(Supplementary Table 1). The isolates were studied for their staining behaviors. The Gram's and Spore staining were performed.

Molecular identification of bacterial isolates

The primer used for the 16S rRNA gene amplification gave 1324 bp length of PCR product and from the product, we obtained approximate 1230 bp good sequencing results (Fig. 2). As per the NCBI blast, the isolated bacteria were identified as *Bacillus altitudinis* and *Bacillus licheniformis*.

Purification of xylanase enzyme

Partial purification of xylanase enzyme by ammonium sulphate precipitation and Dialysis

Partial purification of the enzyme was carried out by the ammonium sulphate precipitation method. The pellet obtained from isolating *Bacillus altitudinis* at 60% saturation has 6.3785 specific activity, 2.3092 folds, and 10.4197% yields (Supplementary Table2), while, the pellets obtained from isolating *Bacillus licheniformis*

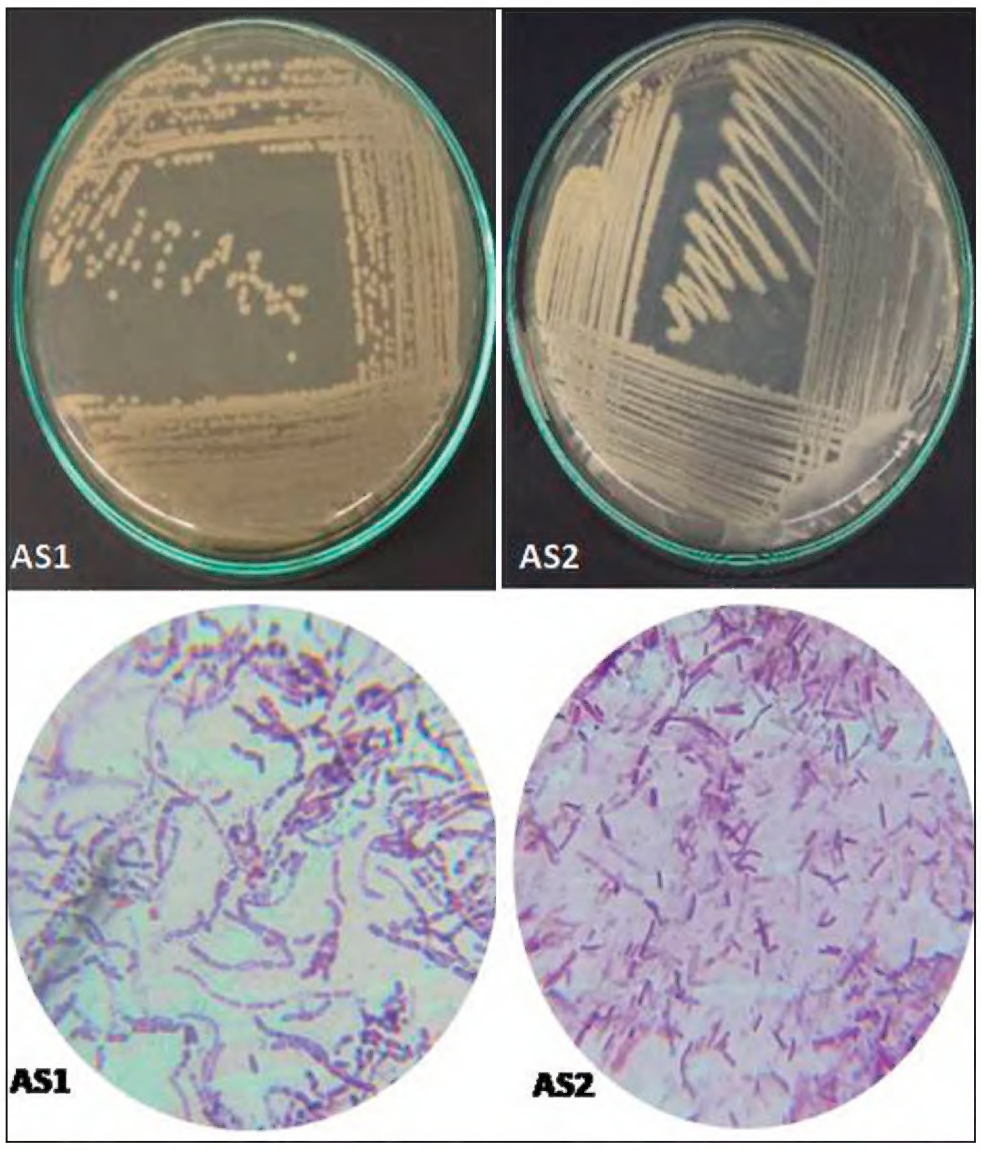

Fig 1. Morphological and gram characteristics of isolated *Bacillus* sp.

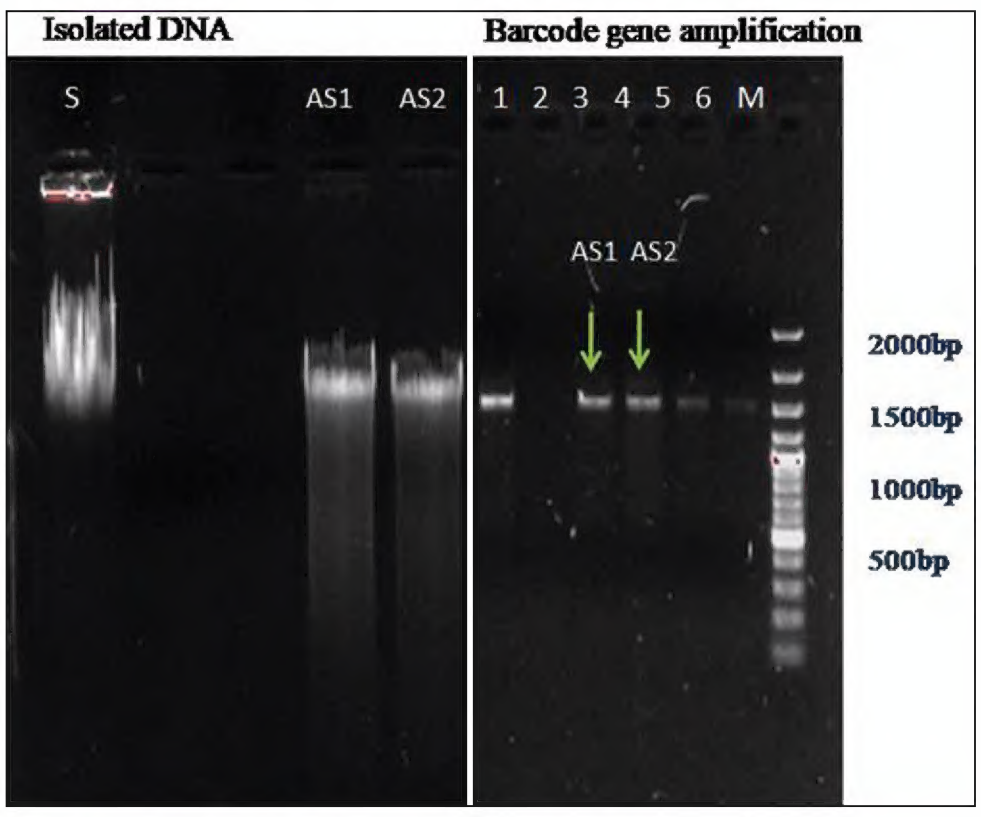

Fig 2. Agarose gel image of isolated DNA and amplified DNA.

at 80% saturation have 6.024492 specific activities, 2.261676 folds, and 1.211778 % yield (Supplementary Table 3). The results indicate higher the 60% saturation and 80% saturation of pellets obtained from isolates *Bacillus altitudinis* and *Bacillus licheniformis* are significant for the partial purification of xylanase. After the purified crude enzyme by ammonium sulphate precipitation method was dialyzed against 3 mM sodium acetate buffer (pH-3.5).

Purification of xylanase by size exclusion chromatography

The partially purified sample of xylanase was loaded on the column and a fraction of 1 ml was collected and analyzed. Fraction 16 of xylanase from *Bacillus altitudinis* showed 25.958 specific activity, 9.3978 folds purity, and a % yield of 2.17754. Fraction 21 of xylanase from *Bacillus licheniformis* showed 27.23593 specific activity, 10.22474 folds purity, and a % yield of 1.294271. The yield is less and needs to be improved. Purification summary of xylanase enzyme from *Bacillus altitudinis* and *Bacillus licheniformis* presented in (Supplementary Table 2 and 3).

Molecular Mass Determination by SDS-PAGE

Performing SDS-PAGE for purification of the enzyme showed a single band on SDS-PAGE. The molecular weight of purified xylanase was 43kDa for AS1 and 40kDa forAS2 (approx.) (Fig. 3).

Characterization of purified xylanase

Effect of pH on xylanase activity

The Xylanase produced by these bacteria showed enzyme activity in a broad range of pH, ranging from pH 3.0 to 8.0. Xylanase from *Bacillus altitudinis* showed maximum activity (309.77 U/ml) under an optimum pH of 5.0, while xylanase from *Bacillus licheniformis* showed the highest enzyme activity (353.68 U/ml) at an optimum pH value of 4.0 (Fig. 4). Further the significance for the pH, Duncan multiple range test with the performed for identifying the significance level of tested parameters (Table 1). The lowest xylanase enzyme activity was observed at pH-8 in both bacteria. These results described the acidic nature of purified enzymes from bacteria *Bacillus altitudinis* and *Bacillus licheniformis*, which have a good enzymatic capability in acidic conditions.

Similar results of optimum pH for xylanase activity at pH-5 were reported from *Bacillus licheniformis* JK7(Seo et al. 2013) and *Bacillus tequilensis* (Khusro et al. 2016). The highest activity of purified Xylanase from *Bacillus altitudinis* Kd 1 at pH-5 was observed (Sharma et al. 2017). Although acid-stable xylanase enzymes are produced by other microbes, such as *Aspergillus flavus* MTCC 9390, *Laetiporus sulfurous*, *Penicilliumoccitania*, *A. niger, Bisporasp*. MET-1, *A. pullulans* (Bhushan et al. 2015; Lee et al. 2009; Driss et al. 2011; Deng et al. 2006; Luo et al. 2009; Ohta et al. 2001).

Effect of temperature on Xylanase activity

The effect of temperature on xylanase enzyme activity from isolated bacteria was determined at different temperatures ranging from 37°C to 60°C. Maximum xylanase activity from isolated bacteria *Bacillus altitudinis* and *Bacillus licheniformis*

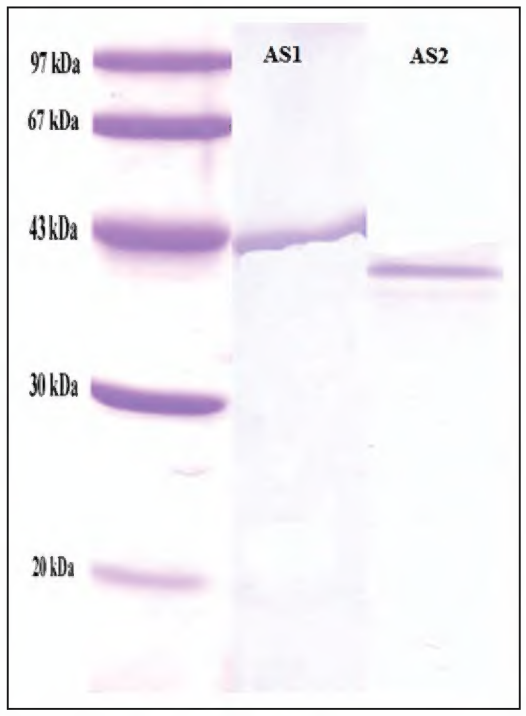

Fig 3. SDS profile of purified xylanase.
(Lane 1 marker, Lane 2 AS1 purified xylanase, Lane 3 AS2 purified xylanase).

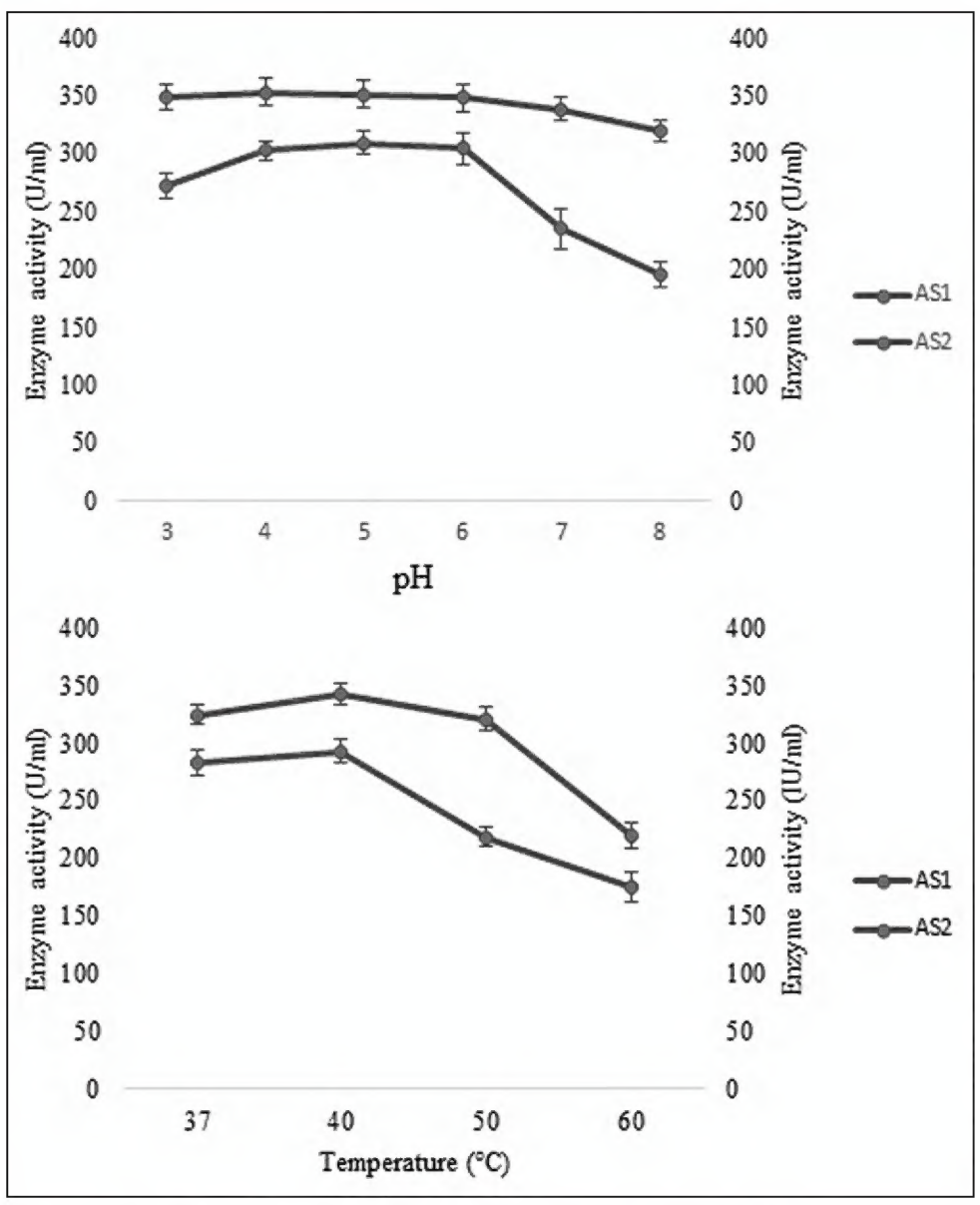

Fig 4. Effect of pH and temperature on xylanase activity.

were detected at the same temperature 40°C and the lowest enzyme activity were observed at 60°C. The results are summarized in Fig.4. Further to determine the significant xylanase activity against different temperature, Duncan multiple range test with the performed for identifying the significance level of tested parameters (Table 2). In addition, the xylanase activity from *P. acidilactici*GC25, *Streptomyces sp.* SWU10, and *Penicillium sp.* CGMCC 1669 was reported as 40°C optimum temperature(Li et al. 2020; Bajaj et al. 2011; Deesukon et al. 2013).

Table 1: Effect of pH on xylanase enzyme activity

Treatment No	pH	Isolate AS1	Isolate AS2
1.	3	1.022^{ab}	0.976
2.	4	1.137^{a}	1.324
3.	5	1.162^{a}	1.327
4.	6	1.142^{a}	1.001
5.	7	0.882^{bc}	1.306
6.	8	0.736^{c}	1.218
CV%		14.08	NS
CD (0.01)		0.29	NS

Means in columns for each index, followed by the same letter, are not significantly different at the $p < 0.01$ level according to Duncan's Multiple Range Test (DMRT).

Table 2: Effect of Temperature on xylanase enzyme activity

Treatment No	Temperature (°C)	Isolate AS1	Isolate AS2
1.	37	1.06^{a}	1.216^{a}
2.	40	1.098^{a}	1.285^{a}
3.	50	0.819^{b}	1.204^{a}
4.	60	0.657^{c}	0.823^{b}
CV%		3.61	5.91
CD (0.01)		0.07	0.14

Means in columns for each index, followed by the same letter, are not significantly different at the $p < 0.01$ level according to Duncan's Multiple Range Test (DMRT).

Effect of metal ions and chelators on xylanase activity

Acid stable xylanase enzymes were assayed in the presence of different metal ions and chelators at 5mM and 10mM concentrations using the substrate as beechwoodxylan. *Bacillus altitudinis* xylanase showed significant activity of 296.08 U/ml and 298.03 U/ml with $MnCl_2$ at 5mM and 10mM concentrations respectively. Metal ions such as $ZnCl_2$, $MgCl_2$, and $CaCu_3$also showed great enzyme activity in comparison to other metal ions. The lowest xylanase enzyme activity was observed with $FeSO_4$ and $COCL_2$ respectively. Xylanase enzyme with the presence of EDTA and SDS, observed activity was maximum at 5mM than 10mM of concentration. Further to determine the significant xylanase activity against different tested metal ions, Duncan multiple range test with the performed for identifying the significance level of tested parameters (Table 3).

Xylanase from *Bacillus licheniformis* showed maximum enzyme activity of 304.436 U/ml and 297.5037 U/ml with $ZnCl_2$ at 5mM and 10mM concentrations respectively. The xylanase enzyme from *Bacillus licheniformis* observed the lowest activity with metal ions $FeCl_3$, $COCl_2$, and $FeSO_4$. The chelators such as EDTA and SDS effect on *Bacillus licheniformis* xylanase were performed. Xylanase with EDTA showed great activity than SDS at 5mM and 10mM (Fig.5). Similarly, it was reported that the xylanase activity from *Bacillus altitudinis* was assayed with 10mM of different metal ions and that was stimulated

Table 3: Effect of metal ions (concentration5mM& 10 mM) on enzyme activity

Sr. No.	Metal Ions	Metal ions (5mM)		Metal ions (10 mM)	
		Isolate AS1	Isolate AS2	Isolate AS1	Isolate AS2
1.	$CaCu_3$	0.809^{c}	0.401^{d}	0.51^{f}	0.468^{e}
2.	$FeCl_3$	0.486^{f}	0.227^{e}	0.46^{g}	0.216^{h}
3.	$CoCl_2$	0.348^{h}	0.347^{d}	0.229^{j}	0.239^{g}
4.	$MgCl_2$	0.88^{b}	0.716^{b}	0.81^{c}	0.686^{b}
5.	$ZnCl_2$	1.083^{a}	1.142^{a}	1.086^{b}	1.116^{a}
6.	$MnCl_2$	1.11^{a}	1.105^{a}	1.118^{a}	1.108^{a}
7.	$CuSO_4$	0.612^{d}	0.552^{c}	0.728^{d}	0.487^{d}
8.	$MgSO_4$	0.53^{e}	0.422^{d}	0.644^{e}	0.442^{f}
9.	$MnSO_4$	0.596^{d}	0.611^{c}	0.452^{g}	0.628^{c}
10.	$FeSO_4$	0.055^{i}	0.117^{f}	0.052^{j}	0.11^{i}
11.	$ZnSO_4$	0.438^{g}	0.428^{d}	0.381^{h}	0.463^{e}
CV%		1.90	10.30	2.70	1.27
CD (0.01)		0.04	0.13	0.03	0.01

Means in columns for each index, followed by the same letter, are not significantly different at the $p < 0.01$ level according to Duncan's Multiple Range Test (DMRT).

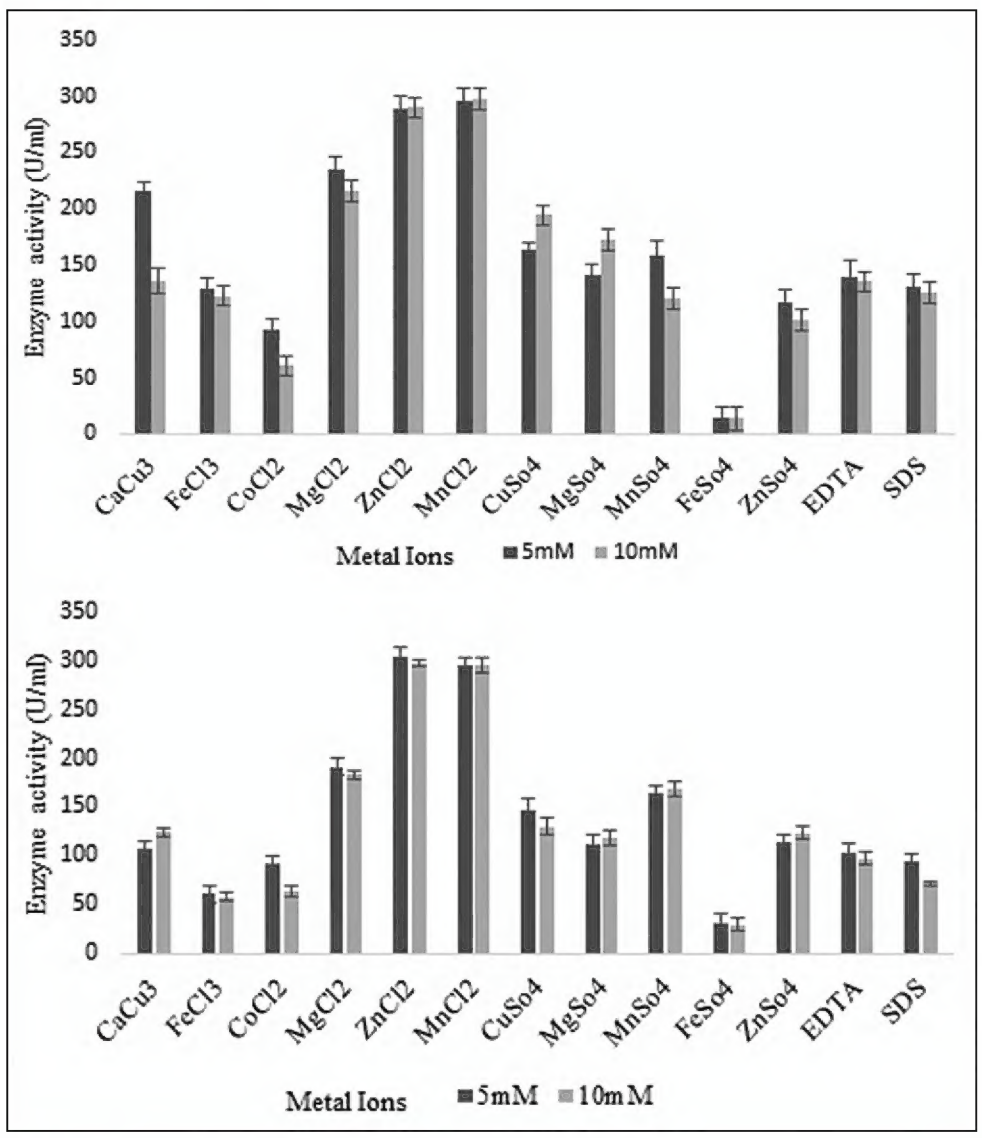

Fig 5. Effect of metal ions on xylanase activity.

enzyme activity in the presence of $MnCl_2$, $CaCl_2$, and $FeCl_3$(Adhyaru et al. 2014).

Application of xylanase enzyme in fruit juice clarification

The viscosity and turbidity of natural fruit juices are due to the presence of polysaccharides such as Pectin, hemicelluloses, cellulose, starch, and bound lignin. Therefore, the degradation of these carbohydrates by suitable enzymes has been promoted for clarification of natural fruit juices (Kumar et al. 2014). Recently, xylan degrading enzymes like xylanase has been used in fruit juice clarification processes (İlgü et al. 2018; Sharma et al. 2017). Many studies are done on the use of pectinase and other enzymes for fruit juice clarification but hardly some literatureis available on the use of xylanase for clarification of fruit juices (Butt et al. 2008). Several studies have reported the xylanase enzyme effect on the clarification process of fruit juices (Nagar et al. 2012; Adiguzel andTuncer 2016; Cakmakand Saglam 2016; Olfa et al. 2007).

In the present study, the purified xylanase enzyme from *Bacillus altitudinis* and *Bacillus licheniformis* was researched on different fruit juice clarification of Apple, Orange, Mousambi, Pineapple, and Kiwi by measuring at 37°C for 1 to 24 hours (Fig. 6). *Bacillus altitudinis* xylanase of 100 ul treatment for 1-hour, Maximum clarity observed was 25.11 %, 75.82%, 52.40%, 40.09, and 30.21% for Apple, Orange, Mousambi, Pineapple, and Kiwi Juice, respectively. Fruits clarification treatment with xylanase from *Bacillus licheniformis* showed maximum clarity was 41.30%, 80.12%, 72.11%, 33.00%, and 21.42% for Apple, Orange, Mousambi, Pineapple, and Kiwi juice, respectively (Table 4 and Table 5). In the case of Apple, Orange, and Mousambi, fruit juice clarification occurred rapidly in comparison to Pineapple and Kiwi fruit juice. The results indicated that fruit juice turbidity decreases with xylanase enzyme application and after 24 hours of incubation, fruit juices become almost clear. Results are summarized in (Table 5). The citrus fruit juice clarification by xylanase resulted in a reduction of 35.34% turbidity was reported (Dhiman et al. 2011). An Orange juice clarification rate of 25-27% with xylanase enzyme has been reported (Algan et al. 2021). Also, the xylanase enzyme from *Geobacillusvulcani* GS90 was reported to clarification of Apple and Orange fruit juice (Algan et al. 2021).

After xylanase enzyme treatment, the total reducing sugar of fruit juices has been improved. The treatment of Apple, Orange, Mousambi, Pineapple, and Kiwi with *Bacillus altitudinis* and *Bacillus licheniformis* xylanase enzyme increases the reducing sugar and decreases turbidity of juice. The highest reducing sugar increase is in the case of fruit juice which has the highest hemicellulose content.

The analysis of chemical and physical parameters of fruit juice after the clarification process showed xylanase enzyme treatment improved the clarification of the juice. The results of the Total soluble solid content of fruit juice is shownin Table 6.A slight increase in soluble solid content in all different fruit juice after xylanase enzyme treatment was also observed. Our findings were similar to the previous

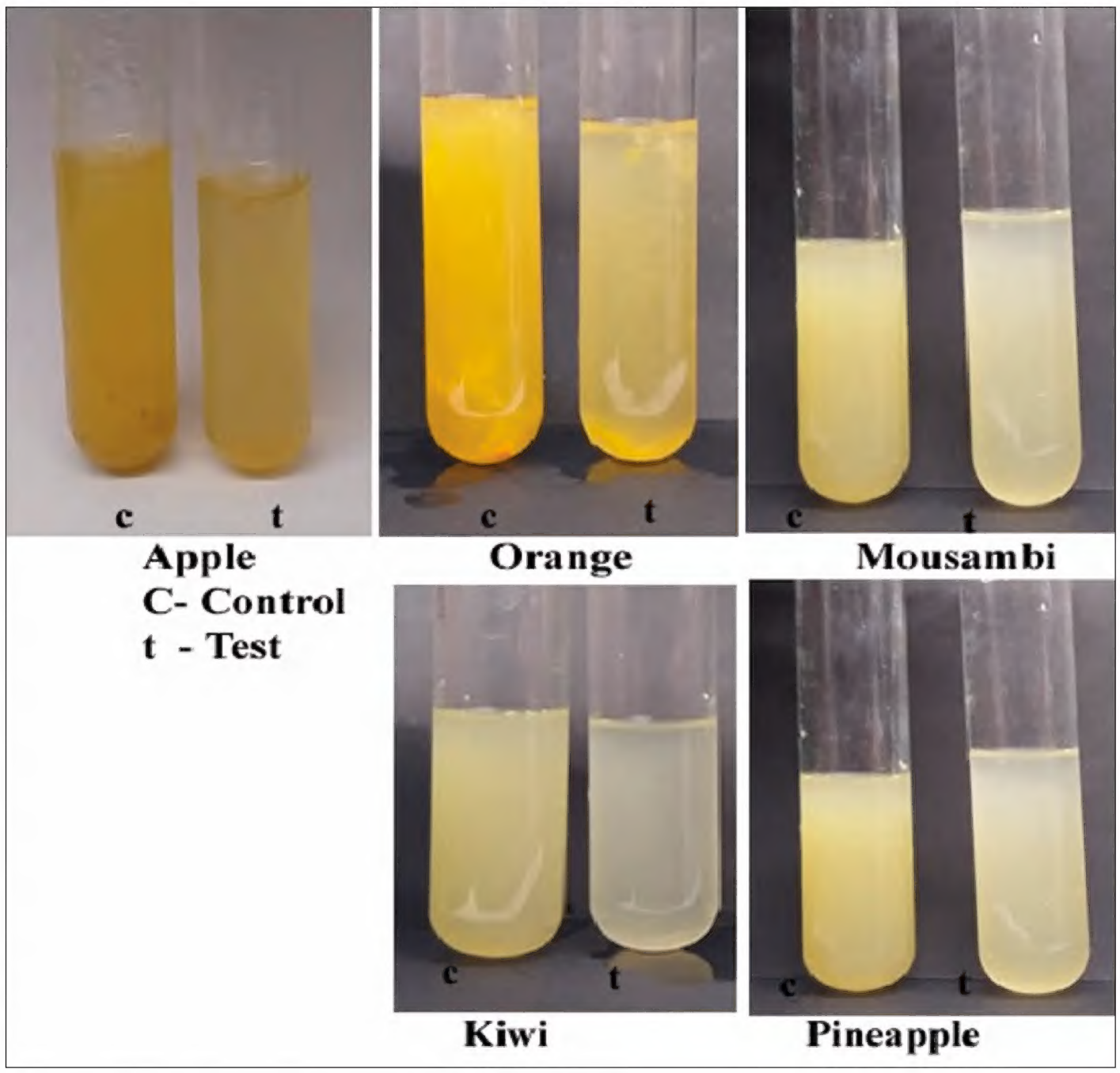

Fig 6. Fruit juice clarification studies using apple, orange, kiwi and pineapple.

Table 4: Fruit juice clarification by xylanase enzyme from *Bacillus altitudinis*

Fruit Juices	Enzyme (U/ml)	Initial turbidity (%)	Control	Clarity (% T) at Time (h)			
				1 h	3 h	6 h	24 h
Apple	50	100	16.21	23.98	23.28	17.33	17.41
	100			25.11	26.04	18.32	17.80
Orange	50	100	46.65	74.20	60.12	56.61	49.05
	100			75.82	61.80	60.32	50.44
Mousambi	50	100	42.16	51.66	51.32	44.12	42.90
	100			52.40	51.64	45.61	44.22
Pineapple	50	100	23.00	39.81	39.08	38.37	25.92
	100			40.09	41.49	38.45	26.12
Kiwi	50	100	13.92	27.71	24.20	18.20	18.09
	100			30.21	27.05	20.96	20.55

Table 5: Fruit juice clarification by xylanase enzyme from *Bacillus licheniformis*

Fruit Juices	Enzyme	Initial turbidity (%)	Control	Clarity (% T) at Time (h)			
				1 h	3 h	6 h	24 h
Apple	50 ul/ml	100	34.25	39.01	42.04	42.15	36.30
	100ul/ml			41.30	44.25	44.31	36.55
Orange	50	100	48.09	76.80	70.00	63.12	51.88
	100			80.12	74.66	68.22	50.08
Mousambi	50	100	41.11	72.10	65.28	62.03	53.41
	100			72.11	66.08	61.00	55.07
Pineapple	50	100	29.15	30.02	32.22	28.12	17.55
	100			33.00	32.25	22.55	17.96
Kiwi	50	100	12.02	21.11	18.32	17.06	17.09
	100			21.42	21.42	17.20	18.52

Table 6: Chemical and physical parameters of fruit juice after clarification using purified xylanase enzyme obtained from *Bacillus altitudinis* and *Bacillus licheniformis*

Fruit Juices	Tested parameters	Control	*Bacillus altitudinis*	*Bacillus licheniformis*
Apple	pH	3.6	3.6	4.0
	Reducing sugar (mg/ml)	12.33	14.39	13.51
	Clarity (%T)	16.21	25.11	44.31
	TSS (in Brix)	7.6	7.9	7.6
	Titratable acidity	0.048	0.041	0.044
Orange	pH	3.4	3.6	3.6
	Reducing sugar (mg/ml)	14.16	16.29	16.44
	Clarity (%T)	46.65	75.82	80.12
	TSS (in Brix)	6.7	5.3	5.1
	Titratable acidity	0.078	0.074	0.074
Mousambi	pH	4.0	4.0	4.1
	Reducing sugar (mg/ml)	12.19	15.01	13.20
	Clarity (%T)	42.16	52.40	72.11
	TSS (in Brix)	6.6	6.8	6.8
	Titratable acidity	0.059	0.055	0.054
Pineapple	pH	3.5	3.5	3.5
	Reducing sugar (mg/ml)	3.37	4.19	3.39
	Clarity (%T)	23.00	40.09	33.00
	TSS (in Brix)	6.2	6.6	6.5
	Titratable acidity	0.065	0.062	0.064
Kiwi	pH	3.3	3.4	3.4
	Reducing sugar (mg/ml)	4.68	4.99	4.92
	Clarity (%T)	13.92	30.21	21.42
	TSS (in Brix)	5.5	6.4	6.9
	Titratable acidity	0.079	0.079	0.075

published reports where the total soluble solid content increase from 5.0 to 5.8 °Brix (Santana et al., 2021).

CONCLUSIONS

Two acidophilic strains *Bacillus altitudinis* and *Bacillus licheniformis* were screened and identified as potential xylanase enzyme producers. Xylanase enzyme was produced under optimized acidic conditions for maximum xylanase production.The highest xylanase activity recorded is 82.664 units per ml and 153.59 units per ml at pH 4.0. Temperature 40°C and pH 4.0 was found to be optimum of purified xylanase enzyme. Additionally, the purified xylanase was active in broad pH range from 3.0- 8.0. The results show that the purified xylanase enzyme can work as potential juice clarifier as it reduces the viscosity of final extracted fruit juice.

ACKNOWLEDGEMENTS

All authors are thankful to Marwadi University for providing infrastructure to carry out the presented research.

CREDIT AUTHOR STATEMENT

Authors contribution in this manuscript is as follows:

Jasmita Chauhan: Conceptualization and Experimentation.
Ashok Kumar Bishoyi: Experimentation (molecular biology part), Statistical analysis, Drafting and editing of Manuscript.
Rajeswaree Gohel: Experimentation and Referencing part of manuscript.
Gaurav Sanghvi: Conceptualization, Supervision, Writing - Original Draft, Writing-Editing.

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SUPPLEMENTARY TABLES

Table 1: Morphological and Microscopic Characteristics of the isolate AS1 and AS2

No.	Characteristics	AS1	AS2
1.	Size	Medium	Medium
2.	Shape	Circular	Irregular
3.	Margin	Entire	Irregular
4.	Elevation	Slightly raised	Flat
5.	Texture	Smooth	Shiny and Moist
6.	Opacity	Opaque	Opaque
7.	Pigmentation	White	Pale White
8.	Odor	Yes	Yes
9.	Gram nature	Positive	Positive
10.	The shape of vegetative cells	Rod-shaped	Rod-shaped
11.	Endospore formation	Yes	Yes
12.	Motility	Motile	Motile

Table 2: Purification summary of xylanase enzyme from *Bacillus altitudinis*

Purification Steps	Enzyme Activity (U/ml)	Protein Conc. (mg)	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude Enzyme	82.66	29.9259	16532	5985.18	2.7621	1	100
Pellet of Ammonium sulphate (60%)	172.26	27.0063	1722.6	270.063	6.3785	2.3092	10.4197
Purified fraction (16)	359.991	13.8681	359.991	13.8681	25.958	9.3978	2.17754

Table 3: Purification summary of xylanase enzyme from *Bacillus licheniformis*

Purification Steps	Enzyme Activity (U/ml)	Protein Conc. (mg)	Total Activity (U)	Total protein (mg)	Specific Activity (U/mg)	Purification fold	Yield (%)
Crude Enzyme	153.5962	57.6621	30719.24	11532.42	2.663729	1	100
Pellet of Ammonium sulphate (80%)	224.2611	37.2249	372.249	372.249	6.024492	2.261676	1.211778
Purified fraction (21)	397.5901	14.598	397.5901	14.598	27.23593	10.22474	1.294271